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BACTERIOLOGICAL PROCEDURE IN SUSPECTED CHOLERA, WITH REPORT OF A POSITIVE CASE.

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Since the beginning of the present epidemic of cholera in Europe the possibility of cases being found on ships arriving at ports in this country has constantly been kept in mind by the Surgeon-General of the Public Health and Marine-Hospital Service. This possibility has been considerably increased by the appearance of the disease in Italy, especially in the city of Naples, which is the point of embarkation for large numbers of immigrants to the United States.

In order that the Hygienic Laboratory might be prepared to render expert aid in the diagnosis of suspected cases of cholera, a supply of cholera agglutinating and also bacteriolytic serum was obtained.

With the use of this serum a positive opinion could be given as to whether organisms isolated from suspected cases of cholera or from suspected carriers were the cholera vibrio. The officers in the Hygienic Laboratory also reviewed the various methods proposed for the isolation and identification of the cholera vibrio in order that when called upon they might be able to render expert aid in the diagnosis of cholera.

The importance of these procedures was shown in the following report on the diagnosis of a case of cholera that appeared in an immigrant who arrived at New York on a ship from Naples and was removed to the quarantine station in accordance with the precautionary methods enforced by Dr. A. H. Doty, quarantine officer.

The suspected organism was isolated from the feces of this person by Dr. E. C. Baldwin, bacteriologist of the New York quarantine. On account of certain cultural and morphological appearances it was suspected of being the cholera vibrio and was brought to the Hygienic Laboratory by Doctor Baldwin in order that agglutination and bacteriolytic tests should be made with it.

The following data as to the patient were kindly furnished us by Doctor Baldwin by letter and are quoted verbatim:

> The patient, Moiazoin Seabain, a Turk, aged 28 years, was removed for observation along with 5 others from the steamship *Germania*, arrived at New York on September 26 (Marseille, September 10, and Naples, September 13). These patients were all treated during the voyage for malaria and were discharged before arrival at this port. When taken from the ship the patient climbed down the ladder and walked into the hospital. On admission it was noted that he was rather weak, with temperature normal. He was given a dose of calomel and the following day, September 27, a dose of salts, the calomel not having taken effect. The next day there were 4 copious

loose movements of the bowels with severe abdominal pain, followed immediately by collapse. The morning and evening temperature on September 28 was subnormal. On September 29, between 8 and 10 a. m., there were several more loose movements, and at 10.30 a. m the patient died.

At autopsy the spleen was found to be much enlarged, but not much softened; the stomach and intestines were congested, particularly the intestines. The Peyer's patches were prominent. The mucosa of the stomach was covered with thick mucus streaked in places with dark blood. The intestines contained a moderate amount of rather thin, yellow fecal matter. The fluid was considerably increased in the pericardial sac, the pleural cavities and also in the meninges and ventricles of the brain. The other organs of the body did not show any pronounced changes.

The material for cultures, etc., was obtained from the stools of September 28, the first that aroused suspicion of the nature of the case.

BACTERIOLOGICAL EXAMINATION OF SUSPECTED CHOLERA VIBRIO CULTURES BROUGHT TO THE HYGIENIC LABORATORY, OCTOBER 3, 1910.

MATERIAL.—The cultures submitted were on 3 per cent salt agar and in peptone solution.

Morphology.—Very actively motile curved rods, apparently in pure culture.

Flagella.—Single, terminal.

Staining.—Stain easily with $\frac{1}{10}$ carbol fuchsin and other dilute dyes. Gram negative.

CULTURAL REACTIONS.—(media neutral to phenol-phthalein).

Bouillon.—Evenly distributed turbidity 18 hours. No precipitate. Delicate grayish pellicle.

Peptone water.—As in bouillon, slightly less turbid.

Agar stab.—Grayish, finely granular needle track growth, round whitish surface growth.

Gelatin stab.—Delicate needle track growth 18 hours. Liquefaction from surface, (22° C.).

Gelatin plates.—Typical, sunken, ground-glass colonies.

Indol production.—Pink color produced in peptone water culture 18 hours old on addition of a few drops of sulphuric acid; cholera-red reaction.

IMMUNITY REACTIONS.—

Agglutination.—Suspension of 18 hours agar slant growth in salt solution, began to agglutinate after 20 minutes at 37° C. and was completely agglutinated in 1 hour at a dilution of 1–2,000 of specific serum having a titre of 1–2,000 with known cholera organisms. Later test with another serum gives agglutination to 1–10,000. Normal serum negative 1–200.

Pfeiffer's reaction.—Bacteriolysis accomplished by a specific cholera bacteriolytic serum having a titre of 1–10,000, in dilutions of 1–5,000 and 1–8,000, no higher dilutions being tried. This occurred in 20 minutes in the peritoneal cavity of guinea pigs. The protocol of the experiment is as follows:

G. P.	Serum.	Culture.	Injected.	Fluid examined—Result.
			Oct. 4, 1910.	
1	1 c. c. 1–5,000 dilution in bouillon of specific rabbit serum.	1 loop 18 hour-agar slant.	2 p. m.....	2.25 p. m. No motile vibrios. Spherical, and other degenerated forms.
2	1 c. c. 1–8,000 dilution in bouillon of specific rabbit serum.	.....do.....	.....do.....	Do.
3	1 c. c. 1–200 dilution in bouillon of fresh rabbit serum.	.....do.....	2.05 p. m.....	Vibrios active and well preserved.
4	1 c. c. bouillon, no serum.....	.....do.....	.....do.....	Vibrios active and well preserved. Died October 5, 1910, after 21 hours.

IMMUNITY REACTIONS—Continued.

Conclusion.—The organism corresponds in all its peculiarities to the true cholera vibrio. While cultural and morphologic characters can not be regarded as in any sense conclusive, the agglutination and Pfeiffer's test are generally accepted as offering positive evidence of the identity of the organism.

METHOD OF BACTERIOLOGICAL EXAMINATION FOR AND IDENTIFICATION OF THE ORGANISM OF ASIATIC CHOLERA, FOR PUBLIC HEALTH AND QUARANTINE PURPOSES.

Suitable materials for examination are the feces, obtained if necessary by an enema of sterile water, or the vomitus, from suspected cases, or intestinal contents from various portions of the ileum, particularly the last portion, obtained at autopsy from the dead body.

The media used in cultural work should be neutral to phenolphthalein.

The material is first plated out on previously poured, hardened, and dried 3 per cent agar, by smearing it out on several successive plates by means of a sterile bent glass rod. Mucous flakes from the intestinal contents should be selected, if possible, for this purpose. These plates are incubated over night at 37° and suspicious colonies examined for morphology and motility in stained smears and the hanging drop. Colonies of active vibrios are then tested for agglutination by emulsifying a portion of the colony in a drop of diluted specific serum, with the platinum needle, using as a control a ten times as strong concentration of normal serum from the same animal species. Colonies not agglutinating should not be discarded but sown on agar slants for further tests, since agglutination of freshly isolated cholera vibrios does not always occur. This is the method giving the quickest results, but since it may often fail the material must also be put through an enrichment process.

The fresh material, about 1 c. c. in amount, is added to 100 c. c. of warm peptone solution in a flask, which is incubated at 37°. After 6 hours a loop is taken from the surface without jarring the flask and examined microscopically. If active vibrios are present agar plates are made with loops from the surface, incubated and examined as in the case of fresh material. If no vibrios are found in 6 hours repeat the examination in 12–24 hours.

A positive agglutination at this stage, controlled as above, justifies a tentative diagnosis of cholera, but the isolated colonies must be fished to agar slants for further examination, as should also colonies of active vibrios which fail of preliminary agglutination.

The pure cultures of active vibrios on agar slants are now to be examined morphologically, culturally, and biologically. The cholera vibrio reacts in the following manner to these tests, always with the understanding that the biological tests (agglutination and Pfeiffer's test) take precedence:

Actively motile, curved rods, nonspore-bearing, having terminal flagellum, easily stained by $\frac{1}{10}$ cold carbol fuchsin, Gram negative, causing uniform turbidity in bouillon and peptone solution with delicate pellicle formation, liquefying gelatin from the surface of stab, or on plates so as to depress the colony, producing indol in peptone solution without the addition of nitrite, agglutinated by specific serum at or near the maximum titre, responding to the bacteriolytic test with specific bacteriolytic serum by Pfeiffer's method properly controlled.